Benchtop Testing Supporting Feasibility to Conduct *In Vivo* Studies of Synthetic Turf/Recycled Tire Crumb Rubber (Abstract 2417)

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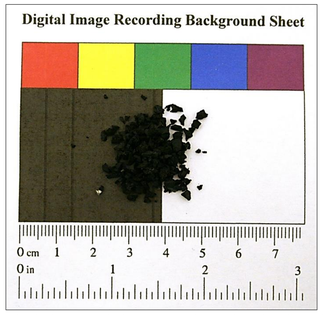
Introduction

Over 12,000 synthetic turf fields exist in the US, with up to 1200 added annually. A primary component of synthetic turf is crumb rubber (CR) infill, which is derived from recycled automotive tires containing potential carcinogenic and toxic substances. However, the potential for human exposure from playing on these fields is not well understood. As such, likely exposure scenarios in humans that could be translated into exposure routes for *in vivo* testing were evaluated. Benchtop trials were conducted to evaluate the challenges associated with dose administration/exposure of CR that would be encountered while performing *in vivo* studies via probable non-inhalation routes of human exposure (dermal and oral). This benchtop testing was performed to determine/support the feasibility of conducting bedding, feed, oral gavage, and dermal exposures.

Test Material

Approximately 10 kg of CR was received from National Institute for Environmental Health Sciences (NIEHS) for testing and stored refrigerated throughout testing.

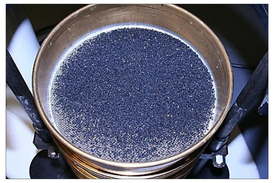


Due to the physical characteristics of CR (irregular sized particles of ground tires that encompass a large size range and composition), physical manipulation methods were performed prior to testing. Milling was not feasible due to the characteristics of rubber (elasticity and thermal properties) and the additional additives employed during the grinding process. Therefore, CR was sieved into various particle sizes for evaluation into each exposure scenario.

During sieving the remainder of the nylon cord fibers that make up the tires agglomerated and formed bundles in the material. These bundles were removed prior to use.

Sieve Mesh	Particle Size Range (µm)	Intended Use	Amount (g)	Comments
14	Greater than 1410	Inhalation, Feed, Bedding (Full Particle Size)	6999.01	NA
40	420 – 1410	Feed, Bedding (Reduced Particle Size)	2848.31	NA
80	170 – 420	Gavage, Dermal	38.71	~75% of nylon cord bundles
400	37 – 170	Gavage, Dermal	33.24	NA
Pan	Less than 37	NA	0.27	All nylon cord bundles

14 Mesh



40 Mesh

80 Mesh

400 Mesh

Objectives

Benchtop trials were conducted to evaluate the challenges associated with dose administration/exposure of CR that would be encountered while performing *in vivo* studies via probable non-inhalation routes of human exposure (dermal and oral). This benchtop testing was performed to determine/support the feasibility of conducting bedding, feed, oral gavage, and dermal exposures. The following formulation combinations were tested for the various routes of exposure.

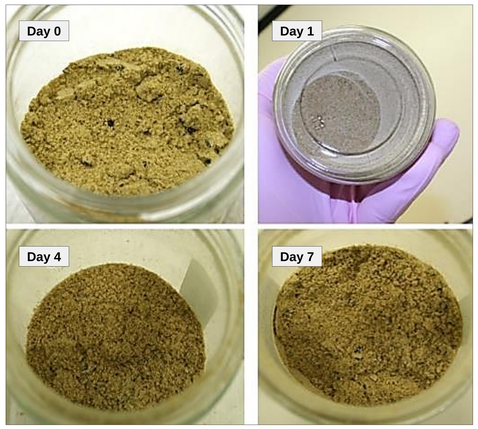
Route of Exposure	Crumb Rubber Mesh	Formulation Vehicle	Target Concentration
Dermal	80	95% Ethanol	300 mg/mL
	400	95% Ethanol	300 mg/mL
Gavage	80	Corn Oil or 0.5% Aqueous Methylcellulose	200 mg/mL
	400	Corn Oil or 0.5% Aqueous Methylcellulose	200 mg/mL
Bedding	14	Bedding (Sani-Chips®)	50:50 w:w
	40	Bedding (Sani-Chips®)	50:50 w:w
Feed	14	Irradiated NTP-2000 Feed	50,000 ppm
	40	Irradiated NTP-2000 Feed	50,000 ppm

Feed Results

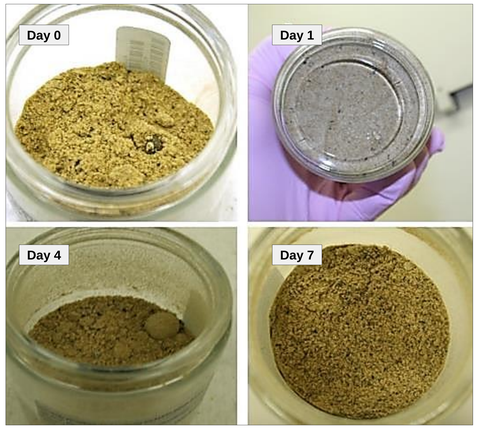
•Mixtures of CR (using 14 or 40 mesh) and irradiated NTP-2000 feed (Zeigler) were prepared at 50,000 ppm CR. Mixtures were formulated in individual glass jars to prepare the appropriate concentration consistently and to preserve homogeneity.

•Jars containing either blank feed, formulation, or formulation with rodent urine and feces (5% w:w) added were rotated on an orbital shaker for 60 minutes/day at ambient temperature for seven days to evaluate uniformity under simulated animal room conditions.

•No visible differences in homogeneity were observed regardless of the particle size of CR used.



14 Mesh

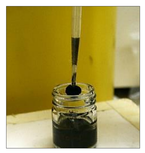


40 Mesh

Dermal Results

- Suspensions of CR (using 80 or 400 mesh) were prepared at 300 mg/mL CR in 95% ethanol. Formulations were mixed vigorously for 15 minutes using stir bar and plate then for 5 minutes using a Silverson mixer.
- Both particle size formulations produced non-homogeneous mixtures containing clumps of CR that could not be drawn into pipettes or syringes for administration.
- Clumping due to volatile nature of ethanol (or other suitable solvents) used for dermal studies that created enhanced surface interactions that caused CR to agglomerate.
- Formulations not feasible for *in vivo* studies.

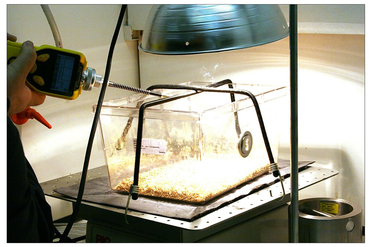
80 Mesh



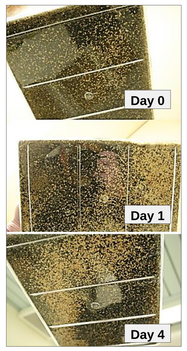
400 Mesh

Bedding Results

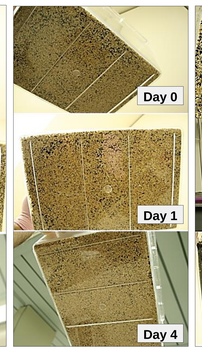
- The use of CR in place of bedding was deemed not feasible with the amount of material available. Due to the non-absorptive nature of CR, multiple cage changes would be required throughout the day in order to maintain an adequate living environment for the animals. A 50:50 (w:w) CR:bedding mixture was deemed to be the maximum amount of CR that could be used in cages in order to maintain the animals' living environment while minimizing the total amount of CR material used for the study.
- Mixtures of CR (using 14 or 40 mesh) and bedding (Sani-Chips®) were prepared at 50:50 (w:w) CR:bedding. Mixtures were formulated in individual animal cages to prepare the appropriate concentration consistently and to preserve homogeneity.
- Cages containing either bedding only, formulation, or formulation with rodent urine and feces (5% w:w) added were rotated on an orbital shaker for 30 minutes/day (ambient temperature) and 15 minutes/day (approximately 31ºC) for 4 days to evaluate uniformity and potential vapor offgassing under simulated animal room conditions. Vapors were monitored with a VOC monitor periodically throughout shaking process.



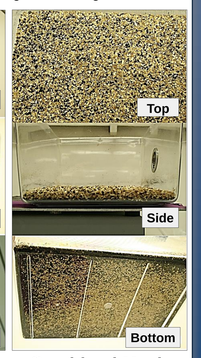
- Elevated VOCs were not observed at any point during testing.
- CR from formulations using 40 mesh CR settled to bottom of cage instantaneously on Day 0 and remained settled out throughout testing, rendering the formulation unsuitable for use.
- Formulations using 14 mesh CR visually maintained homogeneity throughout testing. Formulations using combined CR for single day uniformity visually maintained homogeneity throughout testing.



40 Mesh



14 Mesh



Combined Mesh

Gavage Results

- Suspensions of CR (using 80 or 400 mesh) were prepared at 200 mg/mL CR in either 0.5% aqueous methylcellulose or corn oil. Formulations were mixed vigorously for 15 minutes using stir bar and plate then for 5 minutes using a Silverson mixer.
- 80 mesh CR was too large to fit through the gavage needle.
- Methylcellulose formulations produced non-homogeneous mixtures containing clumps of CR that could not be drawn into pipettes or syringes for administration.
- Corn oil formulations using 400 mesh CR produced a homogeneous suspension that was gavageable.
- Formulation must be taken up into syringe without needle, but can be dispensed through gavage needle within 7 seconds (1 mL).
- Corn oil formulations are stable and resuspendable when stored refrigerated for up to 7 days. Formulations had a thicker consistency but were still dispensable through gavage needle within 10 seconds (1 mL).

80 Mesh Methylcellulose

400 Mesh Corn Oil Uptake with Needle

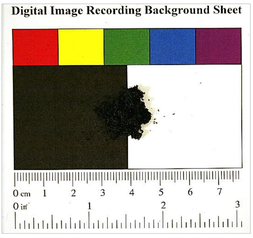
400 Mesh Corn Oil Dispensing

Conclusion

•Following preliminary testing, the 14, 40, and 80 mesh fractions were combined (known as combined mesh) and the bedding formulation uniformity confirmed with the combined CR fraction for one day. The following CR fractions were used for toxicity studies:

Sieve Mesh	Particle Size Range (µm)	Intended Use	Amount (g)	Comments
Combined (Greater than 80 mesh)	Greater than 170	Inhalation, Feed, Bedding	8327.37	--
400	37 – 170	Gavage	27.44	--
Pan	Less than 37	NA	0.27	All nylon cord bundles

Combined



400 Mesh

•Dermal formulations cannot be prepared as homogeneous suspensions using the procedures tested.

•Gavage formulation can be prepared as homogeneous suspensions in corn oil using only the 400 mesh material at concentrations up to 200 mg/mL. The formulations can be stored refrigerated in glass jars for up to 7 days. The formulation must be pulled up into the syringe prior to attachment of the gavage needle and administration.

•Bedding formulations can be prepared as visually homogeneous formulations using the combined material in a 50:50 w:w CR:bedding mixture when formulated in individual animal cages at the time of use (to prepare the appropriate concentration consistently and to preserve homogeneity).

•Feed formulations can be prepared as visually homogeneous formulations using any size material at concentrations up to 50,000 ppm when formulated in individual glass jars and transferred to individual feeders at the time of use (to prepare the appropriate concentration consistently and to preserve homogeneity). The combined material will be used for future toxicity studies.

Acknowledgments

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